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(54) Title: METHODS AND MATERIALS RELATED TO BIOADHESIVE CONTRACEPTIVE GELS

(57) Abstract

The present invention relates generally to materials and methods for contraception as well as restoring the protective acidic environment of the vagina via the administration of the disclosed bioadhesive gel compositions.

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METHODS AND MATERIALS RELATED TO BIOADHESIVE CONTRACEPTIVE GELS

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to contraception, and more specifically, to bioadhesive chitosan-containing gels and their use as contraceptive agents.

Related Technology

Contraceptive methods currently available and employed today have definite undesirable characteristics. For example, the most effective and popular contraceptive methods, *i.e.*, contraceptive hormones, sterilization, and intrauterine devices provide no protection against sexually transmitted diseases. In contrast, barrier contraceptive methods such as condoms, diaphragms, and vaginal spermicides help to prevent sexually transmitted disease transmission by interposing a mechanical or chemical barrier between the female and the male (*i.e.*, most importantly between the uterine cervix and the glans and urethral orifice of the penis). This barrier action is effective in preventing sexually transmitted diseases because secretions from the foregoing sites (*i.e.*, cervical mucus and semen) are the most important sources of sexually transmitted diseases pathogens and also for the fact that these sites are the most susceptible targets for many of the same pathogens. However, the present barrier methods suffer from poor acceptance and/or poor efficacy because inconvenience of use, and/or have undesirable chemical side-effects. Specifically, male and female condoms are cumbersome to use and have been blamed for reducing sexual pleasure and intimacy for either the male or female or both. Further, and most importantly, commercially-available vaginal spermicides, either used alone or in conjunction with other barrier methods (male or female condom) have been shown to erode the vaginal mucosa if used too frequently, and even with infrequent use, may disrupt the protective normal vaginal flora. Such disruption of the normal vaginal flora renders the user susceptible to a number of sexually transmitted diseases.

Therefore, there remains a need for an improved contraceptive method that not only effectively prevents pregnancy but also effectively and safely maintains the normal and protective flora, thereby prevent the transmission of sexually transmitted diseases as well as the induction of other disease states induce by the destruction of the normal vaginal flora (*e.g.*, vaginitis, vaginosis, and urinary tract infections).

SUMMARY OF THE INVENTION

The present invention is directed to bioadhesive gel compositions comprising chitosan and citric acid. The compositions may be used in methods for contraception, killing spermatozoa, or inactivating spermatozoa. In another aspect, the invention is directed to methods for producing bioadhesive gel compositions comprising chitosan and citric acid.

As yet another aspect, the present invention is directed to methods for restoring the acidic environment of the vagina via the administration of the disclosed bioadhesive gel compositions. The invention is further directed to methods for alleviating the loss of the acidic environment of the vagina. The present invention is also directed to methods of restoring the natural acidic protective flora of the vagina.

Other objectives and advantages of the invention may be apparent to those skilled in the art from a review of the following detailed description, including any data present herein, as well as the approved claims.

DETAILED DESCRIPTION OF THE INVENTION

The development of barrier contraceptives is a field of research receiving growing attention as of late. Methods of contraception that a woman is capable of personally controlling are greatly desired. An ideal contraceptive would be one that is (1) easily acquired and administered, (2) inexpensive, (3) lacks systemic effects, (4) lacks the ability to cause irritation to the user or her partner, (5) maintains normal flora and pH of the vagina and, (6) provides contraceptive effects. Current contraceptive methods provide some, but not all of these requirements. For example, hormonal contraceptives, while convenient to use, have systemic effects and require regular visits to clinicians for the monitoring of possible severe side-effects (*e.g.*, edema, weight

gain, abdominal bloating, nausea, depression, acne, hirsutism, vaginal bleeding, and adverse effects on plasma lipoprotein profiles). Barrier contraceptives such as the diagram and cervical caps require fitting by a clinician and concomitant use of spermicides, *i.e.*, nonoxynol-9. Nonoxynol-9, which is the most common spermicide in use today, has been shown to cause irritation in a large segment of the user population. The use of nonoxynol-9 also causes the destruction of the normal vaginal flora, leaving the user susceptible to sexually transmitted diseases as well vaginal disease states or conditions (see below) that normally flourish during an increase of the vaginal pH.

The pH of the vagina of a healthy female is in the acidic range (pH 3.5-4.5) and is generated and maintained by the production of lactic acid by *Lactobacilli*, which form a major component of the healthy vaginal flora. Together with other factors, this acidic pH is widely recognized as preventing overgrowth of undesirable endogenous microbes (*Candida*, harmful anaerobes, and other bacteria that may cause urinary tract infections) and encourages the continued dominance of *Lactobacilli* which, in addition to mild acidity, provides other protective mechanisms such as production of hydrogen peroxide.

It is also known that sperm are inactivated by the mild acidity of the vagina of a healthy female, and acid substances have been used as home made vaginal contraceptives for centuries. More recently it has been recognized that many sexually transmitted disease pathogens such as *Neisseria* gonorrhea [McCutchan *et al.*, *Infection and Immunity*, **15**:149-155 (1977)], *Treponema pallidum*, *Haemophilus ducreyi*, and most or all enveloped sexually transmitted viruses [Kemp *et al.*, *Transfusion*, **31**:423-427 (1991); Martin *et al.*, *J. Infectious Disease*, **152**:400-403 (1985)] including herpes simplex virus, cytomegalovirus, and human immunodeficiency virus, are also inhibited or inactivated by mild acidic pH. However, despite the acidic environment of the vagina, semen contains a potent alkaline buffering capacity that neutralizes the vaginal acidity for a period of many hours following intercourse. This alkaline buffering capacity enables sperm to travel from the vagina into the cervix and ultimately to the upper reproductive tract of the female.

Unfortunately, sexually transmitted disease pathogens in genital secretions can also exploit this period of neutral vaginal pH, since it allows time for them to reach and infect their target cells. If this semen-induced neutralization of vaginal acidity could be promptly and reliably overcome, both contraception and sexually transmitted disease prevention could be achieved by a method that closely mimics the normal physiological state of the vagina.

Maintaining an acidic vaginal environment during menstruation would also have benefits. During menses, the protective vaginal acidity is temporarily lost due to the rate at which neutral and strongly buffering menstrual fluids enter the vagina. Consequently, acid inhibition of deleterious vaginal flora (such as *Staphylococcus aureus*, *Candida albicans*, harmful anaerobes, and other bacteria that may cause urinary tract infections) is lost for a period of 4-7 days each menstrual cycle.

Elevated pH also allows certain strains of *Staphylococcus aureus* to produce toxic shock toxin I, whereas production of this toxin is completely inhibited at a pH of less than 5 [Schlievert *et al.*, *J. Infectious Disease*, **147**:236-242 (1983)]. Thus, loss of protective acidity may result in staphylococcal toxic shock syndrome, *Candida* vaginitis, bacterial vaginosis, or urinary tract infection. Reestablishing vaginal acidity may be therapeutic in reversing established vaginitis (*Candida* and *Trichomonas* vaginitis) and the non-inflammatory condition termed bacterial vaginosis (bacterial vaginosis is characterized by an elevated vaginal pH due to reduction in *Lactobacilli* populations and increase in other vaginal anaerobic bacteria).

Acid pH is also inhibitory to the harmful anaerobes whose overgrowth is associated with the malodorous discharge of bacterial vaginosis. Attempts have been made to treat these conditions with acidifying gels, with some success [*e.g.*, Holst *et al.*, *J. Infectious Disease*, **22**:625-626 (1990)]. However, the effectiveness of these products is limited by their limited buffering capacity, and the fact that they may drip out of the vagina and the acidifying agent may be absorbed across the vaginal mucosa. The clinical results indicate that a method that provides greater acidic buffer capacity, and that used a buffer that could be fully retained in the vagina would improve the therapeutic performance of this method for treating common vaginal infections.

Despite the recognition that acidic buffering of the vagina could protect against many sexually transmitted diseases and other deleterious vaginal conditions, an appropriate vaginal buffering strategy has not previously been devised. Many buffers that might be employed would be ineffective due to their toxicity. The acidifying power required is large (approximately 0.5 milliequivalent of HCl to acidify a 5 ml ejaculate or 5 ml blood to below pH 5), and many types of acidic buffers would be excessively hypertonic and/or caustic if employed in sufficient dose. Second, many possible buffers are small molecules that rapidly leave the vagina by diffusing through the vaginal mucosa, thus limiting the duration of protection they can provide.

Third, the crucial importance of proper positioning of the buffering agent so that it forms a protective barrier between the uterine cervix and the penile urethra has not previously been recognized. The cervix is an anatomic site of great vulnerability not only because it is the portal for fertilizing sperm, but because the cervix is the primary target for many sexually transmitted disease pathogens. If infected, both the penile urethra and uterine cervix are primary sources of sexually transmitted disease pathogens.

The bioadhesive gel of the present invention has been developed with the foregoing in mind. Specifically, chitosan ([β-(1-4)-2-amino-2-deoxy-D-glucan] is a partially deacetylated biopolymer of N-acetyl glucosamine, which has been widely used in biomedical applications due in part to its low toxicity in humans. It is a natural, water-soluble, derivative of chitin. Chitosan is manufactured primarily from chitin obtained from exoskeletons of crustaceans (crabs and crayfish).

Several functional properties of chitosan make it attractive for use as a topically applied gel. Chitosan contains several amine groups giving it a strong positive charge, unlike most other polysaccharides. The strong positive charge allows solutions of chitosan to form films on or to directly adhere to negatively charged surfaces, such as skin. Such formed films (i.e., bioadhesive gels) would most likely not only act as carriers for the spermicidal agent, but would easily coat the walls of the vagina thereby preventing transmission of sexually transmitted diseases. At low pH chitosan is soluble in water dissolving to form a gel. The low pH of chitosan gel is compatible with the naturally low pH of the human vagina.

The invention is illustrated by the following examples, which are not intended to limit the scope of the invention as recited in the claims.

Example 1 describes the preparation of the different formulations of bioadhesive contraceptive gels.

Example 2 describes the effect of the bioadhesive contraceptive gel on the growth of vaginal flora.

Example 3 describes the effect of the bioadhesive contraceptive gels on the buffering capacity of semen and sperm motility.

Example 4 demonstrates the anti-viral effects of the bioadhesive gel on a feline herpes virus.

Example 5 describes the effect of the bioadhesive gel on animal breeding.

Example 6 demonstrates the effects of chronic administration of the bioadhesive gel.

Example 7 describes the ability of the bioadhesive gels to (1) maintain the natural vaginal flora in human females and (2) act as a spermicide.

Example 8 describes the use of bioadhesive gels in the treatment of vaginal conditions related to an increase in vaginal pH.

EXAMPLE 1
PREPARATION OF BIOADHESIVE CONTRACEPTIVE GEL

A bioadhesive contraceptive gel of the present invention was prepared according to the following protocol. Chitosan (CTC Organics, Atlanta, GA) was subjected to a preliminary purification. Specifically, the chitosan was dissolved in 2% acetic acid (Mallinckrodt, Chesterfield, MO) with constant stirring, overnight. To remove any insoluble material the solution was filtered using a 20 μm screen (Tetko, Inc., Briarcliff Manor, NY) and the pH of the filtrate was adjusted to 7.75-8.25 with the addition of 5N sodium hydroxide, which caused the dissolved chitosan to precipitate. Precipitated chitosan was then washed 5 times with deionized water. The resultant mixture, consisting of a wet paste of chitosan (approximately 4% to 5.2% of chitosan) and water, was stored at 4°C until needed.

As an initial step in producing the bioadhesive gel, approximately 3-6 grams of the chitosan paste use dried for 1-2 hours at 100° C (using a standard drying oven) to a white powder. The powder was used in the determination of percent dry weight of chitosan and discarded. After determination of the percent dry weight, 3 M sterile citric acid (10X solution; solid citric acid, anhydrous USP obtained from Spectrum Quality Products, Gardena, CA) was added to the chitosan paste in an amount sufficient to achieve a final concentration of 0.3 M and mixed until the chitosan paste began to dissolve. Once the chitosan began to dissolve, the paste was further diluted with 0.3 M sterile citric acid to a final concentration of chitosan of 2%, (based upon the percent dry weight previously determined). The resultant chitosan mixture was left at room temperature overnight to dissolve and form into a gel (having a pH of about 1.5 to about 3.5). Upon complete formation, the bioadhesive gel was autoclaved for 15 minutes on liquid cycle.

Although the present example is exemplified by production of a 2% chitosan gel in 0.3 M citric acid, the percentage of chitosan in the bioadhesive gel may range from 1% to 5% and the final concentration of citric acid in the gel may range from .1 M to 1 M. Further, the bioadhesive gels described above may also include pharmaceutically acceptable excipients, stabilizers, diluents, and/or preservative (for example, but not limited to, benzalkonium chloride, boric acid, or succinic acid), which are to be used in concentrations that do not interfere with the activity of the contraceptive gels described herein.

EXAMPLE 2
EFFECT OF BIOADHESIVE CONTRACEPTIVE GEL ON GROWTH OF VAGINAL FLORA

As discussed above, flora naturally present in the vaginal environment produce acidic conditions that prevent sexually transmitted pathogens from infecting one or both partners as well as prevent harmful conditions related to an increase in vaginal pH. Therefore maintenance of the normal vaginal flora is critical for maintaining a healthy vaginal environment. In order to assess the effect of the chitosan bioadhesive

contraceptive gel on the natural flora of the vagina, two 1.5% chitosan / 0.5 M citric acid bioadhesive gels were produced as described above in Example 1. Included within two formulations of the gel were two different concentrations (0.1% or 0.5%) of benzalkonium chloride (BZK; Sigma Chemical Co., St. Louis, MO). BZK was included in the event it was necessary to use a preservative in the bioadhesive gel to extend the shelf-life of the gel. Although bacteriostatic/bactericidal (depending on the concentration) by itself, experiments were conducted to determine the proper concentration of BZK to enable its use as a preservative rather than as a bacteriostatic/bactericidal agent.

In order to assess their effects on vaginal flora, each formulation of bioadhesive gel was spread over a different chocolate agar plate (100 µl/plate) and allowed to dry for ten minutes (control plates did not contain a bioadhesive gel). Vaginal swabs were obtained under sterile conditions from normal, healthy non-menstruating women and were spread on the plates. Plates were incubated for 48 hours at 37°C under anaerobic conditions and subsequently visually analyzed for microbiological vaginal flora growth. Results, shown in Table 1, indicate that the formulation of bioadhesive contraceptive gel (1.5 % chitosan / 0.5 M citric acid) allowed normal vaginal flora growth as compared to the baseline control. These results also indicated that both of the concentrations of BZK used (0.1 % or 0.5%) were detrimental to the normal growth of vaginal flora (0.1%, growth decreased by 25%; 0.5% growth decreased by greater than 50%).

TABLE 1

Gel Formulation	Vaginal Flora Growth
none (control)	baseline
1.5% chitosan / 0.5 M citric acid gel	normal
1.5% chitosan / 0.5 M citric acid gel with 0.1% BZK	growth decreased by 25%
1.5% chitosan / 0.5 M citric acid gel with 0.5% BZK	growth decreased by <50%

Similar experiments were conducted using a different formulation of the bioadhesive gel. Specifically a 2% chitosan /0.3 M citric acid gel was produced as described in Example 1. Again, included within two formulations of the gel were two different concentrations of BZK (0.01% and 0.1%). For this experiment, normal vaginal flora were obtained from one healthy women at the mid-point of her estrus cycle. As described above, swabs of the normal vaginal flora were cultured on chocolate agar plates for 48 hours at 37°C under anaerobic conditions. Another set of plates were then prepared with pure cultures of five different bacteria, which are represented in the normal vaginal flora (*i.e.*, *Lactobacilli*, *Staphylococci*, and three different unidentified gram negative rods). These secondary cultures (pure) were also incubated for 48 hours at 37°C under anaerobic conditions. Paper disks were soaked in either (1) a bioadhesive gel of 2% chitosan / 0.3 M citric acid, (2) a bioadhesive gel of 2% chitosan / 0.3 M citric acid plus 0.01% BZK, (3) a bioadhesive gel of 2% chitosan / 0.3 M citric acid plus 0.1% BZK, or (4) a commercial contraceptive product containing the spermicide nonoxynol-9 (Advantage24™, Lake Consumer Products, Vernon Hills, IL). Control cultures were grown without exposure to a gel.

Results (obtained by visual analysis) showed that the product containing nonoxynol-9 and the bioadhesive gel containing 0.1% BZK were detrimental to the growth of the *Lactobacilli*, while the bioadhesive gel containing 0.01% BZK was shown not to be detrimental to the growth of the *Lactobacilli*. The *Staphylococci* were shown to be slightly sensitive to the gel alone, somewhat sensitive to the gel containing 0.01% BZK, very sensitive to the bioadhesive gel containing 0.1% BZK, and not sensitive at all to product containing nonoxynol-9. With respect to the different gram negative rods, one was sensitive to both the bioadhesive gel containing 0.1% BZK and to the product containing nonoxynol-9, while the second gram negative rod was sensitive to the gel formulation containing BZK, while the third gram negative rod was not sensitive to any of the formulations used.

These results indicate the bioadhesive gel alone or the bioadhesive gel + 0.01% BZK did not disrupt the normal vaginal flora, while the bioadhesive gel + 0.1% BZK

or the product containing nonoxynol-9 were detrimental the growth of normal vaginal flora.

EXAMPLE 3
EFFECT OF BIOADHESIVE CONTRACEPTIVE GELS ON BUFFERING CAPACITY OF SEMEN AND SPERM MOTILITY

As discussed above, sperm are secreted with a buffering capacity to counteract the acidic environment of the vagina. In the following set of experiments different formulations of a bioadhesive gel are tested for the ability to destroy the buffering capacity of the sperm, thereby rendering them non-motile. All semen samples were collected from volunteers via masturbation and allowed to liquefy for 30 minutes. A modified Sander-Cramer test [Sander *et al.*, *Human Fertility*, **6(5)**:134 (1941)] was used to evaluate the spermicidal effect of various gel formulations tested.

A. Bioadhesive Gels Formulated in Different Acids

In the first set of experiments, various bioadhesive gels were produced in order to test the ability of the different formulations to reduce sperm motility, thus increasing the gel's contraceptive effect. The following bioadhesive gel formulations were produced as described above in Example 1 (except that acids other than citric acid were tested): (a) 1% chitosan / 2% acetic acid; (b) 2% chitosan / 0.5% formic acid; and (c) 1.5% chitosan / 0.5 M citric acid. Under sterile conditions, 100 μl of the bioadhesive gel (to be tested) was added to 100 μl of semen. Immediately following the addition of the bioadhesive gel, the semen samples were microscopically examined for spermicidal activity. Gels were defined as being spermicidal (*i.e.*, contraceptive) if sperm motility ceased either immediately or within a few minutes following the addition of the bioadhesive gel. Results, shown in Table 2 indicated that the chitosan/citric acid bioadhesive gel render the sperm non-motile immediately upon addition of the gel, *i.e.*, this formulation apparently eliminated the buffering capacity of the semen. Preparations of gels using either acetic acid or formic acid were shown to have no spermicidal effects. Specifically, when mixed with semen these formulations were unable to overcome the buffering capacity of the semen (*i.e.*, the

buffering capacity of the semen was not completely eliminated, thus the final pH was alkaline enough for the sperm to survive).

Table 2

Gel Formulation	pH of semen sample prior to addition of gel	pH of gel prior to addition to semen sample	pH of semen sample / gel mixture	Motility of sperm
1% chitosan / 2% acetic acid	7.5	4.0	5.5	normal (up to fours hours after the addition of the gel)
2% chitosan / 0.5% formic acid	7.5	3.7	6.0	normal (up to fours hours after the addition of the gel)
1.5% chitosan / 0.5 M citric acid	7.5	2.7	3.5	non-motile (immediately upon addition of gel)

B. 2% Chitosan / 0.5 M Citric Acid Bioadhesive Gel

A 2.5% chitosan / 0.5 M citric acid bioadhesive gel, having an adjusted pH of 3.1, was produced as described above in Example 1 and tested for its ability to act as a spermicide at differing gel volumes (this formulation was used to establish parameters to determine dosage for use in clinical trials). Specifically, the modified Sander-Cramer test used herein involved mixing semen with various concentrations of contraceptive agents and observing sperm microscopically for motility. Under sterile conditions, varying amounts (see Table 3) of the 2.5% chitosan / 0.5 M citric acid bioadhesive gel were added to 100 μl of semen (collected as described above). Immediately following the addition of the bioadhesive gel, the semen samples were microscopically examined for spermicidal activity. Results obtained (see Table 3) demonstrate that at 0.05, 0.1, and 0.05 mls of gel, the gels were spermicidal on contact. With respect to the administration of 0.01 mls of bioadhesive gel, the sperm were rendered totally non-motile approximately 2.5 minutes after addition of the gel. In these experiments a preparation was considered to have contraceptive activity if all sperm within a sample were non-motile immediately after mixing the semen sample and gel samples.

Table 3

Volume of Gel (mls)	Volume of Semen (mls)	pH of gel/semen mixture	Motility
0.5	0.1	3.5	non-motile immediately upon contact
0.1	0.1	4.0	non-motile immediately upon contact
0.05	0.1	4.5	non-motile immediately upon contact
0.01	0.1	4.75	rendered completely non-motile by 2.5 minutes

Overall these results indicate that even at low gel volume 1 (0.05 ml), the bioadhesive gel of the present invention is a potent spermicide and thus a useful contraceptive.

C. 2% Chitosan / 0.3 M Citric Bioadhesive Gel

A 2% Chitosan / 0.3 M citric acid gel, having a pH of 2.4, was produced as described in Example 1 and tested for its ability to act as a spermicide at differing dilutions of the gel (modified Sander-Cramer test). Under sterile conditions, varying dilutions (see Table 4) of the 2 % Chitosan /0.3 M citric acid bioadhesive gel were added in an equal volume to 100 μl of semen (collected as described above). Immediately following the addition of the bioadhesive gel, the semen samples were microscopically examined for spermicidal activity. Results from these experiments (see Table 4) show that the bioadhesive gel had immediate spermicidal activity at up to a 1:3 dilution. Further, at a 1:6 dilution complete non-motility occurred at three minutes, while at a 1:8 dilution complete non-motility did not occur for approximately 15 minutes.

Table 4

Gel Dilution	Semen Volume	Motility	pH of final mixture
no dilution	100 μl	immediate non-motility	3.5
1:2	100 μl	immediate non-motility	3.75
1:3	100 μl	immediate non-motility	4.0
1:6	100 μl	non-motile after 3 minutes	4.5
1:8	100 μl	non-motile after 15 minutes	5

Overall the results of the foregoing tests studies demonstrate that if the buffering capacity of semen was not eliminated, the final pH remained alkaline enough for sperm to survive. Without the ability of the gel to eliminate the buffering capacity of semen no spermicidal effect is seen.

EXAMPLE 4
ANTI-VIRAL EFFECTS OF BIOADHESIVE CONTRACEPTIVE GEL

Cat kidney cells (CRFK; American Type Culture Collection Accession No. CCL-94; Manassas, VA) were plated (Multiplicity of Infection, 1) in 96 well tissue culture plates and allowed to attach and grow to 85% confluence for 24 hours at 37°C in a CO_2 incubator. For the initial attachment and growth phase, cells were cultured in Modified Eagle Medium (MEM; GIBCO-BRL, Gathersburg, MD) and 5% Fetal Bovine Serum (FBS; Hyclone, Logan, UT). Following 24 hours, the growth media was replaced with one of the following test media:

(1) MEM + 5% FBS, pH 7.4;

(2) MEM + 5% FBS +0.5 M citric acid, pH 6.5 [100 μl/5 ml media; final concentration of citric acid, 10 mM];

(3) MEM + 5% FBS + 2.5% chitosan / 0.5 M citric acid bioadhesive gel, pH 6.95 [100 μl/5 ml of media; final concentration of citric acid, 10 mM, final concentration of chitosan, 0.05%]; or

(4) MEM +5% FBS + 2.5% chitosan / 0.5 M citric acid bioadhesive gel + 5% BZK , pH, 7.0 [100 μl/5 ml of media; final concentration of citric acid, 10 mM, final concentration of chitosan, 0.05%; final concentration of BZK, 0.01%].

Test Media No. 1 was the experimental control, Test Media No. 2 contained citric acid only, Test Media No. 3 contained the bioadhesive gel, while Test Media No. 4 contained the bioadhesive gel and the preservative, BZK.

In order to test the anti-viral activity of the bioadhesive gel, CRFK cells were incubated with feline rhinotracheitis virus (a feline herpesvirus; American Type Culture Collection Accession No. VR-815; ATCC, Manassas, VA). Specifically, stocks of the

feline virus were diluted (10^2) in each of the foregoing test media (1-4), and 30 μl of the dilution was added to the cells and incubated for 1 hour at 37°C. After incubation with the feline virus, the test media was removed and replaced with the same test media but that did not contain the feline herpesvirus. Following the replacement of media the plates were cultured for an additional 48 hours and microscopically examined for cytopathic effects. Cytopathic effects were defined as a foci of disruption of cellular growth (caused by infection of the feline herpesvirus). Results, set forth in Table 5, indicate that the bioadhesive gel (Test Media No. 3) had a greater anti-viral activity against the feline herpesvirus than either control (Test Media No. 1) or application of citric acid alone (Test Media No. 2). No foci were noted when Test Media No. 4 was used as the addition of the BZK into the MEM + 5% FBS media caused a precipitate to form that covered the cells.

Table 5

Test Media	Average No. of Foci Per Well	n	Number of Foci Per Test Well
1	59	2	Well No. 1, 68 foci; Well No. 2, 50 foci.
2	10.75	4	Well No. 1, 7 foci; Well No. 2, 9 foci; Well No. 3, 14 foci; Well No. 4, 13 foci.
3	3.25	4	Well No. 1, 3 foci; Well No. 2, 4 foci; Well No. 3, 2 foci; Well No. 4, 4 foci.
4	n/a	4	no cytopathic effects noted

Means were compared using the student t-test at $\alpha=0.005$, and were shown to be statistically significant.

The foregoing experiment was repeated as described above. The results again indicated the bioadhesive gel (avg. foci per well, 51) had a significant anti-viral effect against the feline herpesvirus as compared to control (avg. foci per well, 172) or citric acid (avg. foci per well, 126). Another experiment was also conducted using a 2% chitosan /.3 M citric acid bioadhesive gel, with equivalent results as set forth above. Cells exposed to the chitosan/citric acid bioadhesive gel show a decrease in infectivity,

meaning that when cells are exposed to virus, fewer foci of cytopathic effects are seen. Not only are fewer cells infected when the gel is present, but there is a retardation of development of cytopathic effects that do occur. This indicates that the presence of the bioadhesive gel causes a decrease in the level of viral infectivity as well as a decrease in the proliferation of the virus.

EXAMPLE 5
EFFECT OF BIOADHESIVE GEL ON ANIMAL BREEDING

In order to determine the *in vivo* contraceptive effectiveness of a the bioadhesive gel, rabbits were administered, intravaginally, a bioadhesive gel or other commercial contraceptive gels and allowed to mate. Specifically, a 1.5% / 0.5 M citric acid gel was produced as described in Example, while a second 1.5% / 0.5 M citric gel with the addition of the preservative, BZK was also produced as described in Example 1. Female rabbits (adult females) were administered (intravaginally) 1 ml of either foregoing bioadhesive gel or one of two commercial contraceptive gels containing the spermicide, nonoxynol-9 [Koromax™ (Schmid Laboratories, Sarasota, FL) or Advantage24™]. Control animals received no treatment. Males were introduced into the cages and the animals were allowed to mate at-will. Results, shown in Table 6, indicate that the 1.5% chitosan / 0.5 M citric acid bioadhesive gel had a positive contraceptive effect as compared to control or the commercial product, Koromax ™, while being slightly less effective than the commercial product, Advantage24™. Further, results with the bioadhesive gel plus BZK indicated that formulation having 0.5% of BZK had a contraceptive effect.

Table 6

Compound Tested	Pregnancy Rate
none	100% (2/2)
Koromax™ (3% nonoxynol-9)	100% (1/1)
Advantage24™ (3.5% nonoxynol-9)	17% (1/6)
1.5% chitosan / 0.5 M citric acid gel	33% (1/3)
1.5 % chitosan / 0.5 M citric acid gel + 0.5% BZK	0% (0/3)

A parallel set of experiments was also conducted wherein the bioadhesive gels were prepared in the manner described in Example 1, but with formic acid (0.25%; chitosan 1.5%) rather than citric acid. Results (data not shown) indicate that the bioadhesive gel prepared with formic acid did not eliminate the buffering capacity of the semen and therefore did not show a contraceptive effect.

EXAMPLE 6
EFFECT OF CHRONIC ADMINISTRATION OF BIOADHESIVE CONTRACEPTIVE GEL

A. Chronic administration of Bioadhesive Gel

Animal test subjects (rabbits or rats) were used to determine if chronic administration of the bioadhesive contraceptive gels of the present invention would have a deleterious effect on the lining of the vagina as well as the cervix. Bioadhesive gels were formulated as described in Example 1 (1.5% chitosan / 0.5 M citric acid), either with or without 0.5% BZK. Formulated gels were sterilized via autoclave and were applied intravaginally 3 times/week for two months. After cessation of the study, animals were sacrificed and tissues were examined for effects of chronic administration.

In the first set of experiments rabbits (four month-old females; two animals per test group) were administered, intravaginally, either a bioadhesive gel alone or in

combination with BZK. Control animals received saline. Results failed to show either external or internal vaginal irritation from either formulation of the bioadhesive gel. With respect to histological changes, no changes to the cervical tissue were noted, while histological changes were noted in the vaginal tissue of one animal (noted as thick, white discharge; on a microscopic level, white cell infiltration).

In a second set of experiments rats (females, 300g; six animals per test group; Harlan-Sprague/Dawley) were administered, intravaginally, either bioadhesive gel alone or in combination with BZK. Control animals received saline. No external nor internal vaginal irritation was seen with either formulation of the bioadhesive gel. Histological examination of vaginal sections indicated no pathological changes.

B. Adjuvant Effect of Bioadhesive Contraceptive Gel

A potential use of chitosan is as an adjuvant to produce or increase an immune response to a given antigen. With respect to the present use of chitosan (in a bioadhesive contraceptive gel), the presence of female antibodies to a partner's sperm would be an unacceptable side-effect of a temporary contraceptive method. Thus the following experiments were conducted to determine if use of the bioadhesive contraceptive gel of the present invention would cause the development of serum (IgG) or mucosal (IgA) antibodies against sperm deposited in the vagina during intercourse.

Rats were used to investigate the adjuvant potential of a bioadhesive contraceptive gel according to the invention. Female rats (250g, Harlan-Sprague/Dawley; n=2) were immunized (via subcutaneous injection, shoulder) with rat sperm using an adjuvant system containing chitosan as an active ingredient (animals immunized by this method should develop serum antibodies to rat sperm functioning as positive controls). A second group of animals (n=2), was administered a mixture of rat sperm and bioadhesive contraceptive gel (4% chitosan/0.5% citric acid) intravaginally, 4 times for a period of 1 week (this group served as the test group). The control group of animals received neither the chitosan-adjuvant system/sperm mixture nor the bioadhesive gel/sperm mixture. Six weeks after administration of the various mixture, serum samples were collected. Rat sperm was solubilized by a standard solubilization protocol and Western analysis was performed

using the collected serum samples as probes. Results indicated that only those animals injected with chitosan- adjuvant system/sperm had mounted an immunological response to rat sperm, while no anti-sperm antibodies were produced in animals exposed to the sperm/bioadhesive gel combination intravaginally. Protocols were repeated to see if a boosting effect could be detected. Again, only animals injected with chitosan-adjuvant system/rat sperm mixture were shown to develop anti-sperm antibodies.

In order to measure levels of mucosal antibodies, saliva was collected from rats in each treatment group. Pilocarpine, a cholinomimetic (Sigma Chemical Co., St. Louis, MO; 200 mg/animal) was administered (via intraperitoneal injection) to the animals to induce excess salivation of each treatment group. The saliva was collected and used for the detection of anti-sperm antibodies by Enzyme-Linked Immunsorbent Assay (using a polyclonal antibody to rat sperm). No animal, even those immunized with the chitosan-adjuvant system/sperm mixture developed mucosal antibodies to sperm.

The foregoing results suggest that chronic intravaginal administration of the bioadhesive contraceptive gel would not cause an individual to mount an immune response to a partner's sperm.

EXAMPLE 7
USE OF THE BIOADHESIVE GEL IN HUMANS AS A CONTRACEPTIVE

In view of the foregoing results, studies on the use of bioadhesive gels according to the present invention were conducted with human subjects. Although the bioadhesive gel used in the present example was formulated as a 4% chitosan / 0.5 M citric acid gel, other formulations of the gel may be used successfully as a contraceptive, *i.e.*, the percentage of chitosan may range from 1% to 5% and the final concentration of citric acid may range from .1 M to 1 M. Finally, the final formulation of bioadhesive gel may contain a preservative or preservatives (to extend shelf-life) in concentrations that do not cause additional contraceptive effects (*e.g.*, BZK at final concentrations at or below 0.01%).

Specifically, a 4% chitosan / 0.5 M citric acid bioadhesive gel was produced as described above in Example 1. Two formulations were produced: the first bioadhesive gel (pH 2.5) did not contain a preservative (pH 2.5, while the second bioadhesive gel (pH 3.5) contained the preservative, BZK (0.05% final concentration). Eleven sexually-active women were divided into two groups. Group A received the bioadhesive gel formulation that did not contain BZK, while Group B received the gel formulation that contained BZK. The study was conducted in two parts. The first part of the study was to determine the effect of the bioadhesive gel on the normal vaginal flora. Results from this study indicate that the pH of the vagina continued to remain acidic after the five day administration of either gel formulation (see Table 7). This portion was conducted by (1) obtaining baseline vaginal cultures from each test subject, (2) administering one dose (2 ml) of the bioadhesive gel, intravaginally, each day for five days, and (3) obtaining a vaginal culture 24 hours subsequent to the administration of the last dose. Microbiological analysis (data not shown) of the vaginal cultures comparing flora before administration of the gel and after administration of the gel revealed that neither formulation caused a disruption in the flora normally found in the vagina of a healthy human female. Further, as shown in Table 7, the pH of the vagina continued to remain acidic after the five-day administration of either gel formulation.

One interesting result was noted with respect to Test Subject No. 10, in that the bioadhesive gel acted as a therapeutic to return the vaginal pH to a normal level. Specifically, the vaginal pH of this test subject prior to administration of the gel was 8.0. Upon microbiological analysis (prior to gel administration) it was noted that the major bacteria present was *Garderella vaginalis*, which has been know to cause vaginitis due its ability to increase the vaginal pH. After the five-day administration of the gel, the vaginal pH in that test subject had returned to more normal acidic pH of 6.0 and microbiological analysis revealed no trace of *Garderella vaginalis*.

TABLE 7

Test Subject	Test Group	Vaginal pH Prior to Gel	Vaginal pH Subsequent to Gel
1	B	5.5	5
2	B	5	4
3	B	6	6
4	B	5	6
5	B	5.5	5
6	B	5.5	4
7	A	5	4.5
8	A	4	5.5
9	A	4	5
10	A	8	6
11	A	5	no data

Vaginal pH prior to gel determined on day 0. Vaginal pH subsequent to gel determined on day 6.

The second part of the study was used to determine whether the bioadhesive gel acted as a spermicide when used intravaginally by human females. Two weeks after the first part of the study was complete, either formulation of the bioadhesive gel was administered (2 ml) intravaginally and immediately prior to sexual intercourse. Within two hours after intercourse, vaginal fluid/ejaculate samples were obtained and examined for the presence of motile/non-motile sperm as well as sperm viability (as determined by Eosin-Blue staining). Results (see Table 8) showed that either formulation of bioadhesive gel was very effective in immobilizing the sperm (100% non-motility).

TABLE 8

Test Subject (Treatment Group)	pH	Motile	Non-motile	Viable	Non-viable
1. (B)	6.5	0%	100%	85%	15%
2. (B)	5.0	0%	100%	0%	100%
3. (B)	5.0	0%	100%	85%	15%
4. (B)	5.5	0%	100%	85%	15%
5. (B)	6.0	0%	100%	70-80%	20-30%
6. (B)	6.0	0%	100%	0%	100%
7. (A)	no data	no data	no data	no data	no data
8. (A)	5.0	0%	100%	0%	100%
9. (A)	6.8-6.9	0%	100%	80-90%	10-20%
10. (A)	5.0-6.0	0%	100%	60-80%	20-40%
11. (A)	6.0	0%	100%	85-90%	10-15%

The foregoing results from human test indicate that intravaginal administration of a chitosan/citric acid bioadhesive gel not does not cause a disruption in the natural flora of the vagina (thereby increasing the pH of the vaginal environment), but also may be used successfully as a contraceptive spermicide to prevent fertilization of the ovum.

EXAMPLE 8
USE OF BIOADHESIVE GELS AS THERAPEUTICS FOR THE TREATMENT OF VAGINAL INFECTIONS

Because the gels of the present invention have been shown to maintain or restore vaginal pH and has been shown to restore or maintain normal vaginal flora, the gels of the invention may be used (1) to treat those conditions that require the restoration of the acidic environment of the vagina, (2) to alleviate the loss of the protective acidic environment of the vagina, (3) to restore the natural protective flora of the vagina, (4) or (as discussed above) prevent or alleviate infections such as, but not limited to sexually transmitted diseases.

Specifically, a female diagnosed with a vaginal condition/infection (*e.g.*, vaginitis, vaginosis, urinary tract infections) related to an increase in the vaginal pH is administered (in an effective amount) one of the formulations of bioadhesive gels discussed here (chitosan concentration may range from 1% to 5% and the citric acid concentration may range from .1 M to 1 M) in an amount effective to alleviate the condition and/or infection. Further, the formulation to be administered may also contain a preservative such as BZK.

Although the present invention has been described in terms of preferred embodiments, it is intended that the present invention encompass all modifications and variations that occur to those skilled in the art upon consideration of the disclosure herein, and in particular those embodiments that are within the broadest proper interpretation of the claims and their requirements.

All literature cited herein is incorporated by reference.

CLAIMS

What is claimed is:

1. A bioadhesive gel comprising chitosan and citric acid.

2. A bioadhesive gel according to claim 1, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

3. A bioadhesive gel according to claim 2 wherein the amount of chitosan ranges from about 1% to about 5%.

4. A bioadhesive gel according to claim 3, wherein the amount of chitosan ranges from about 1.5% to about 4%.

5. A bioadhesive gel according to claim 4, wherein the amount of chitosan is 2%.

6. A bioadhesive gel according to claim 2, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

7. A bioadhesive gel according to claim 6, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

8. A bioadhesive gel according to claim 7, wherein the final concentration of citric acid is .3 M.

9. A bioadhesive gel of claim 1 further comprising a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

10. A bioadhesive gel according to claim 9, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

11. A bioadhesive gel according to claim 9, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

12. A bioadhesive gel according to claim 9, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

13. A bioadhesive gel produced by the process of:

(a) dissolving chitosan in an acetic acid solution;

(b) filtering the resulting chitosan solution to remove any insoluble chitosan;

(c) adjusting the pH of the filtrate to precipitate the dissolved chitosan;

(d) washing the precipitate with deionized water to form a water/chitosan paste; and

(e) dissolving the water/chitosan paste with citric acid until a viscous gel is formed.

14. A bioadhesive gel prepared by the process according to claim 13, wherein the pH of the filtrate of step (c) is adjusted from about 7.75 to about 8.25.

15. A bioadhesive gel prepared by the process according to claim 13, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

16. A bioadhesive gel prepared by the process according to claim 15 wherein the amount of chitosan ranges from about 1% to about 5%.

17. A bioadhesive gel prepared by the process according to claim 16, wherein the amount of chitosan ranges from about 1.5% to about 4%.

18. A bioadhesive gel prepared by the process according to claim 17, wherein the amount of chitosan is 2%.

19. A bioadhesive gel prepared by the process according to claim 15, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

20. A bioadhesive gel prepared by the process according to claim 19, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

21. A bioadhesive gel prepared by the process according to claim 20, wherein the final concentration of citric acid is .3 M.

22. A bioadhesive gel prepared by the process according to claim 13, the gel further comprising a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

23. A bioadhesive gel prepared by the process according to claim 22, wherein the pharmaceutically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

24. A bioadhesive gel prepared by the process according to claim 22, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

25. A bioadhesive gel prepared by the process according to claim 22, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

26. A method of contraception comprising placing a contraceptively effective amount of a chitosan/citric acid bioadhesive gel in the vagina of a human female.

27. The method of claim 26, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

28. The method of claim 27 wherein the amount of chitosan ranges from about1% to about 5%.

29. The method of claim 28, wherein the amount of chitosan ranges from about 1.5% to about 4%.

30. The method of claim 29, wherein the amount of chitosan is 2%.

31. The method of claim 27, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

32. The method of claim 31, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

33. The method of claim 32, wherein the final concentration of citric acid is .3 M.

34. The method of claim 26, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

35. The method of claim 34, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

36. The method of claim 34, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

37. The method of claim 34, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

38. A method of killing spermatozoa comprising exposing the spermatozoa to a spermicidally effective amount of a chitosan/citric acid bioadhesive gel.

39. The method of claim 38, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

40. The method of claim 39 wherein the amount of chitosan ranges from about 1% to about 5%.

41. The method of claim 40, wherein the amount of chitosan ranges from about 1.5% to about 4%.

42. The method of claim 41, wherein the amount of chitosan is 2%.

43. The method of claim 39, wherein the final concentration of citricacid ranges from about .1 M to about 1 M.

44. The method of claim 43, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

45. The method of claim 43, wherein the final concentration of citric acid is .3 M.

46. The method of claim 38, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

47. The method of claim 46, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

48. The method of claim 47, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

49. The method of claim 47, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

50. A method of inactivating spermatozoa comprising exposing the spermatozoa to an effective amount of a chitosan/citric acid bioadhesive gel.

51. The method of claim 50, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

52. The method of claim 51, wherein the amount of chitosan ranges from about 1% to about 5%.

53. The method of claim 52, wherein the amount of chitosan ranges from about 1.5% to about 4%.

54. The method of claim 53, wherein the amount of chitosan is 2%.

55. The method of claim 51, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

56. The method of claim 55, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

57. The method of claim 56, wherein the final concentration of citric acid is .3 M.

58. The method of claim 50, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

59. The method of claim 58, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

60. The method of claim 58, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

61. The method of claim 58, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

62. A method for restoring the acidic environment of the vagina comprising placing an effective amount of a chitosan/citric acid bioadhesive gel in the vagina of a human female.

63. The method of claim 62, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

64. The method of claim 63, wherein the amount of chitosan ranges from about 1% to about 5%.

65. The method of claim 44, wherein the amount of chitosan ranges from about 1.5% to about 4%.

66. The method of claim 65, wherein the amount of chitosan is 2%.

67. The method of claim 63, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

68. The method of claim 67, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

69. The method of claim 68, wherein the final concentration of citric acid is .3 M.

70. The method of claim 62, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

71. The method of claim 70, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

72. The method of claim 70, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

73. The method of claim 70, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

74. A method of alleviating the loss of the protective acidic environment of the vagina of a human female comprising placing an effective amount of a chitosan/citric acid bioadhesive gel in the vagina of a human female.

75. The method of claim 74, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

76. The method of claim 75, wherein the amount of chitosan ranges from about1 % to about 5%.

77. The method of claim 76, wherein the amount of chitosan ranges from about 1.5% to about 4%.

78. The method of claim 77, wherein the amount of chitosan is 2%.

79. The method of claim 75, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

80. The method of claim 79, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

81. The method of claim 80, wherein the final concentration of citric acid is .3 M.

82. The method of claim 74, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

83. The method of claim 82, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

84. The method of claim 82, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

85. The method of claim 82, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

86. A method of restoring the natural protective flora of the vagina of a human female comprising placing an effective amount of a chitosan/citric acid bioadhesive gel in the vagina of a human female.

87. The method of claim 86, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

88. The method of claim 87, wherein the amount of chitosan ranges from about 1% to about 5%.

89. The method of claim 88, wherein the amount of chitosan ranges from about 1.5% to about 4%.

90. The method of claim 89, wherein the amount of chitosan is 2%.

91. The method of claim 87, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

92. The method of claim 91, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

93. The method of claim 92, wherein the final concentration of citric acid is .3 M.

94. The method of claim 86, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

95. The method of claim 94, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

96. The method of claim 94, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

97. The method of claim 94, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

98. A method for preventing or alleviating infection comprising placing an effective amount of a chitosan/citric acid bioadhesive gel in the vagina of a human female.

99. The method of claim 98, wherein the pH of the bioadhesive gel ranges from about 1.5 to about 3.5.

100. The method of claim 99, wherein the amount of chitosan ranges from about 1% to about 5%.

101. The method of claim 100, wherein the amount of chitosan ranges from about 1.5% to about 4%.

102. The method of claim 101, wherein the amount of chitosan is 2%.

103. The method of claim 99, wherein the final concentration of citric acid ranges from about .1 M to about 1 M.

104. The method of claim 103, wherein the final concentration of citric acid ranges from about .1 M to about .5 M.

105. The method of claim 104, wherein the final concentration of citric acid is .3 M.

106. The method of claim 98, wherein the bioadhesive gel further comprises a pharmaceutically acceptable excipient, stabilizer, diluent, and/or preservative.

107. The method of claim 106, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is benzalkonium chloride.

108. The method of claim 106, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is boric acid.

109. The method of claim 106, wherein the pharmacuetically acceptable excipient, stablizer, diluent, and/or preservative is succinic acid.

110. A bioadhesive gel comprising chitosan, citric acid, and benzalkonium chloride.

111. A bioadhesive gel comprising chitosan, citric acid, and boric acid.

112. A bioadhesive gel comprising chitosan, citric acid, and succinic acid.

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INTERNATIONAL SEARCH REPORT

In. ational Application No
PCT/US 99/08357

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K31/715 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 474 769 A (SMITH, R.L.) 2 October 1984 (1984-10-02) abstract column 1, line 1 - line 35 column 3, line 1 - line 30 column 3, line 47 - line 51; claims 1-5,9-13,15-18,21 ---	1,26,38, 50
Y	DATABASE WPI Section Ch, Week 9528 Derwent Publications Ltd., London, GB; Class B05, AN 95-207395 XP002900544 & CN 1 085 778 A (ZHANG C), 27 April 1994 (1994-04-27) abstract --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 1999	20. 09. 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mosser

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INTERNATIONAL SEARCH REPORT

In. .ational Application No
PCT/US 99/08357

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 8644 Derwent Publications Ltd., London, GB; Class D21, AN 86-287934 XP002900545 & JP 61 210015 A (SHISEIDO CO LTD), 18 September 1986 (1986-09-18) abstract ---	1
A	WO 95 08981 A (UNION CARBIDE CHEMICALS & PLASTICS TECHNOLOGY CORPORATION) 6 April 1995 (1995-04-06) abstract page 26, paragraph 2; claim 1 ---	1-12, 26-61, 110-112
A	DATABASE WPI Section Ch, Week 9412 Derwent Publications Ltd., London, GB; Class A96, AN 94-097759 XP002900546 & JP 06 048917 A (NAKAGAWA M), 22 February 1994 (1994-02-22) abstract -----	1, 110-112

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/08357

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 62-109 concern methods for treatment of the human body by therapy (PCT-Rule 39.1 (iv)), the search was carried out and based on the alleged effects.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ational Application No
PCT/US 99/08357

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